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Dietary SWF® enhanced growth performance and disease resistance in hybrid sturgeon (*Acipenser baerii x Acipenser schrenckii*) mediated by the gut microbiota

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ABSTRACT

The presence of healthy gut microbiota in the gastrointestinal tract of fish is important for the optimal function of gut, which plays a significant role in the host growth and health. The aim of the present study was to investigate the effect of dietary stress worry free (SWF®) on growth, feed utilization and disease resistance of hybrid sturgeon (*Acipenser baerii x Acipenser schrenckii*). Sturgeon were fed for three weeks with SWF® supplemented or basal diet. The weight gain and FCR of sturgeon fed on the diet supplemented with SWF® were significantly improved ($P < 0.05$). SWF® supplemented diet provoked an increase in the resistance of sturgeon against *A. veronii* Hm091 ($P = 0.09$). In terms of gut microbiota, the number of total bacteria, *Fusobacteria, Firmicutes,* and *Proteobacteria* were increased significantly in the SWF® group ($P < 0.05$), whereas significant reduction of *Actinobacteria* was observed in the gut of the SWF® group compared with the control group ($P < 0.01$). Moreover, at the end of the experiment the gut microbiota of sturgeon, were colonized to germ-free (GF) zebrafish for three days. Results indicated that, the expression of growth promoter genes mTOR, MyoD and Myogenin was significantly higher in GF zebrafish colonized with gut microbiota of SWF® group of sturgeon. Furthermore, TGF-β was increased significantly in GF zebrafish colonized with gut microbiota from SWF® group ($P < 0.01$), whereas the expression of TNF-α was significantly decreased ($P < 0.05$). The expression of non-specific immune related genes DEFBL-1, C3a and Lysozyme was significantly increased in GF zebrafish colonized with gut microbiota of sturgeon fed on SWF® ($P < 0.05$). Group of GF zebrafish colonized with gut microbiota of sturgeon fed on SWF® had significantly higher survival rate against *A. veronii* Hm091 ($P < 0.05$). Our study suggests that, the gut microbiota induced by SWF® played a great role in growth and disease resistance of sturgeon using GF zebrafish model.

Abbreviations: DEFBL-1, Defensin Beta-Like 1; GF, Germ Free; GI, Gastrointestinal; SWF®, Stress Worry Free; IL-1β, Interleukin 1 beta; mTOR, mechanistic Target of Rapamycin; MYoD, Myoblast Determination Protein 1; C3a, Complement 3a; TNF-α, Tumor Necrosis Factor alpha; TGF-β, Transforming Growth Factor -Beta

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1. Introduction

China is the leading country in aquaculture production in the world with annual production of 69.96 million tons in 2017 (China fishery statistical yearbook, 2018). Although aquaculture production in China is growing faster, the cold water aquaculture production is not as fast as like the other aquaculture types. Therefore, cold water aquaculture has a lot of room for improvement in order to use the resource fully. Sturgeon is one of the cold water fish species, most popular and economically important fish in aquaculture due to its higher market price (Li et al., 2009), fast growing (Luo et al., 2015), and tolerance to environmental change (Patterson et al., 2017). Moreover it has a remarkably good nutritional value, especially in 'caviar' (Wang et al., 2017). The hybrid sturgeon (*Acipenser baerii x Acipenser schrenckii*) is one of the dominant farmed sturgeons in China (Wei et al., 2011).

Gut microbiota contributes a great role in activates of many complex systems in the fish physiology. Studies showed that the gut microbiota of fish participate in several complex physiological system of the fish including in metabolic homeostasis, digestive function, stimulating the host immune response, life span, development of the GI tract, and protection against infections (Burns et al., 2016; Li et al., 2019; Nie et al., 2017; Palareti et al., 2016; Piazzon et al., 2017; Smith et al., 2017; Wang et al., 2018; Yan et al., 2016). Plethora studies showed that modulation of the gut microbiota using probiotics and postbiotics plays significant role in the health and function of GI tract of the fish as well as on the fitness of the fish (Aguilar-Toalá et al., 2018; Jaramillo-Torres et al., 2019; Kanwal and Tayyeb, 2019; Kim et al., 2017; Liu et al., 2017; Sanders et al., 2017). Application of *Bacillus subtilis* resulted stimulation of the local and systemic immune system and promoted growth and disease resistance in Nile tilapia (Galagarza et al., 2018; Yu et al., 2019, 2017). Furthermore, *B. subtilis* and *B. cereus* promote intestinal colonization of tilapia (Garcia-marengoni and Menezes-albuquerque, 2016). Currently, in addition to probiotics, postbiotics have been applying in aquaculture (Biswas et al., 2013; Mahmoud A.O. Dawood et al., 2015a; Panigrahi et al., 2010; Zheng et al., 2017). Postbiotics are defined as "soluble products or metabolic byproducts secreted by live bacteria or released after bacterial lysis and they provide physiological benefits to the host" (Aguilar-Toalá et al., 2018). Nowadays, studies clarified that postbiotics have an advantage over probiotics (Aguilar-Toalá et al., 2018; De Marco et al., 2018; Shenderov, 2013). Therefore, it is crucial to increase our knowledge on beneficial gut bacteria, their cell components and metabolites, which colonize the GI tract of the fish, in the context of improved growth performance and health of the fish.

The GF zebrafish (*Danio rerio*) model was successfully established in 2004 (Rawls et al., 2004). The GF zebrafish provides a number of advantages that simplify the generation of gnotobiotic organisms and research the interaction between the host and microbiota and serve as powerful models for revealing the functions of intestinal microbiota (Brugman, 2016; Tan et al., 2019). In addition to this, zebrafish were used as a model to evaluate many diets for aquaculture species (Ulloa et al., 2011). In this study we used GF zebrafish model to evaluate the effect of the dead probiotic bacteria (SWF®) on hybrid sturgeon. The gut microbiota of hybrid sturgeon fed on either the SWF® supplemented or basal diet were transferred to the GF zebrafish which provides the possibility of understanding the influence on host biological processes including gene expression, immunity and disease resistance. We selected *A. veronii* for the challenge test, since *A veronii* is an emerging pathogen causing severe pathology and mortalities in several aquaculture species (Hasan et al., 2019; Smyrli et al., 2019). Therefore, presently modulation of the host gut microbiota using postbiotics has been posited as a possible mechanism involved in the improvement of fish growth, feed utilization, immune regulation, disease resistance against pathogens, and generally the fitness of the fish and can be biotheraptic mechanism in aquaculture.

In this study, we examined the ability of postbiotics (SWF®) on hybrid sturgeon growth, feeding conversion, survival and gut microbiota abundance of hybrid sturgeon. Furthermore, we evaluated the effect of hybrid sturgeon gut microbiota on the expression of growth promoter and immune regulation related genes, and disease resistance against *A. veronii* Hm091 of GF zebrafish model.

Table 1
Ingredient and nutrient composition of basal diet.

Ingredient (g/100 g diet)	Basal feed
Fish meal	63.50
Soybean oil	18.50
Corn flour	12.00
Fish oil	4.00
Soybean phospholipid oil	1.1
Mono calcium phosphate	0.50
Vitamin premix[a]	0.20
Mineral premix[b]	0.20
Total	100.00
Crude protein	60.64
Crude fat	14.24

[a] Containing the following (g/kg vitamin premix): thiamine, 0.438; riboflavin, 0.632; pyridoxine·HCl, 0.908; *d*pantothenic acid, 1.724; nicotinic acid, 4.583; biotin, 0.211; folic acid, 0.549; vitamin B-12, 0.001; inositol, 21.053; menadione sodium bisulfite, 0.889; retinyl acetate, 0.677; cholecalciferol, 0.116; *dl*-α-tocopherol-acetate, 12.632.

[b] Containing the following (g/kg mineral premix): CoCl2·6H2O, 0.074; CuSO4·5H2O, 2.5; FeSO4·7H2O, 73.2; NaCl, 40.0; MgSO4·7H2O, 284.0; MnSO4·H2O, 6.50; KI, 0.68; Na2SeO3, 0.10; ZnSO4·7H2O, 131.93; Cellulose, 501.09.

2. Materials and methods

2.1. Stress worry free (SWF®) and experimental diets

Stress Worry Free (SWF®) provided by Beijing Sino-Norway Joint Aquaculture Technology, Co., Ltd. SWF® is mainly composed of *Bacillus subtilis, Lactococcus lactis* and *Cetobacterium somerae* without live bacteria. These microbial strains originate from the GI tract of fish and stored in Sino-Norway Joint Lab on Fish Gut Microbiota, Feed Research Institute of Chinese Academy of Agricultural Sciences, Beijing 100081, China.

We prepared two types of diets, the basal diet and SWF® supplemented diet. The formulations of basal diet as well as its proximate composition were as shown (Table 1). SWF® supplemented diet was prepared by mixing of 5 g/kg of basal diet. Before adding of the SWF® to the basal diet, it was mixed with appropriate amount of sterile water, then added to the basal diet and mixed evenly. Then leave at room temperature for 30 min before feeding.

2.2. Animals and treatments

All experimental and animal care procedures were conducted in agreement with protocols approved by the Feed Research Institute of the Chinese Academy of Agricultural Sciences Animal Care Committee, under the auspices of the China Council for Animal Care (Assurance No. 2018-AF-FRI-CAAS-001). The experiment was conducted in Sino-Norway Joint Lab on Fish Gut Microbiota, Feed Research Institute, Beijing, China. This study consists of two experiments. The first experiment was focused on the growth performance and modulation of sturgeon gut microbiota by dietary SWF®. The second experiment was conducted after the completion of experiment 1 and the main aims were to evaluate the effect of SWF® induced gut microbiota of sturgeon on the expression of growth promoter and immune regulatory genes, and disease resistance of GF zebrafish.

Experiment 1. Juvenile hybrid sturgeons (*Acipenserbaerii* x *Acipenserschrenckii*) were purchased from Beijing Fisheries Research

Institute and housed in 60 × 40 × 20 cm glass tank recirculating system of Sino-Norway Joint Lab on Fish Gut Microbiota, Feed Research Institute of Chinese Academy of Agricultural Sciences, Beijing, China. Healthy and tidy fry with body size of 7−12 cm were selected and randomly divided into the SWF® group and the control group. The SWF® group was fed on SWF® supplemented diet, whereas the other group fed the basal diet only (used as control) for three weeks. Six replicate tanks were randomly assigned per treatment group within the group 30 fry of hybrid sturgeon were assigned. Fish were fed four times in a day at 4 h interval with 2.5 % of their body weight. During the feeding period, the rearing temperature was 16 °C, the dissolved oxygen was > 6.0 mg/L, the pH was 7.0–7.2, the nitrogen content was < 0.50 mg/L, and the nitrogen content (as NO_2) was < 0.02 mg/L. At the end of the experiment the sturgeon were stocked in a MS-222 solution and then scarified.

Experiment 2. GF zebrafish were prepared following established protocols as described previously (Rawls et al., 2006; Ran et al., 2016). The GF zebrafish were randomly divided in to two groups, each group contain 6 replications. In each replication 30 GF zebrafish were housed. Sturgeons were fed with SWF® diet or the basal diet for three weeks, and then at 4 h post the last feeding, the gut content samples were collected from three fish in each tank and pooled as a replicate. 100 μg of the gut content of sturgeon fed on either experimental diet were placed into 15 mL centrifuge tubes containing 6 mL of sterile phosphate-buffered saline (PBS). After gentle mixing of the gut content with PBS solution and letting stand another 5 min, 200 μL suspensions were added to each tank of 3 days post fertilization (dpf) GF zebrafish. One group of GF zebrafish was cultured in the tank (T25 cell culture bottle, NestBiotechnology, Wuxin, China) contains gut microbiota from the sturgeon fed on the diet supplemented with SWF® and the other group from sturgeon fed on control diet. GF zebrafish were cultured in the tank for three days without feeding. During the culture time GF zebrafish were taken the sturgeon's gut microbiota from the water.

2.3. Growth and survival rate measurements

At end of the experimental period to evaluate the effects of SWF® on weight gain and feeding performance, sturgeons from each group were weighed and calculated according to previous reports (Guo et al., 2017).

Weight gain (WG): [100 × (final body weight-initial body weight)/ initial body weight], feed conversion ratio (FCR): food intake (g)/ weight gain of fish (g); survival rate (SR) = (number of fish at the end of the experiment/number of fish at the start of the experiment) ×100, and daily feeding rate (%/d) = 100 × total feed consumed/[days × (initial body weight + Final body weight)/2]. The fish in each tank were weighed to calculate WG.

2.4. Intestinal contents sampling and bacterial quantitative determination by a 16S rRNA-based qRT-PCR analysis

Sturgeons were fed with SWF® diet or the basal diet for three weeks, and then at 4 h post the last feeding, the gut content samples were collected from six fish in each group and pooled as a replicate. DNA was extracted from each pooled sample using a Fast DNA SPIN Kit for Soil (TianGen, Beijing, China), according to the manufacturer's instructions. The number of total bacteria or a specific phylotype was quantified by *q*RT-PCR (Ludwig and Schleifer, 2000). Primer sets for universal bacteria or specific bacterial groups targeted the 16S rRNA gene and are listed in Table 2, as our previously described (Zhang et al., 2019). 16S rRNA of the universal bacteria was cloned into the pLB vector (Tiangen, Beijing, China) according to the manufacturer's procedure as a copy number standard. For the *q*RT-PCR standard, the copy number concentration was calculated based on the length of the PCR product and the average mass of a DNA base pair. Results were expressed as copy numbers of bacterial 16S rDNA per milligram of intestinal contents.

2.5. Quantitative real-time PCR analysis

Total RNA was isolated from zebrafish larvae and extracted with TRIzol Reagent (Invitrogen, Carlsbad, CA, USA). First-strand complementary DNA synthesis (cDNA) was performed using the Superscript First-Strand Synthesis System (Invitrogen, Carlsbad, CA, USA). Quantitative real-time PCR reaction was performed using the SYBR Green Supermix (TianGen, Beijing, China) on the Light Cycler 480 (Roche 480). The primer sequences were listed (Table 3).

2.6. Bacteria disease resistance

After 3 weeks feeding of sturgeon on either of the experimental diets, 20 hybrid sturgeons from each treatment group with 6 replications were taken for resistance test of highly pathogenic *A. veronii* Hm091. The source of the pathogenic *A. veronii* Hm091 was from Sino-Norway Joint Aquaculture Technology, Co., Ltd, Feed research institute of Chinese Academy of Agricultural Sciences, Beijing, China. For the challenge we used a concentration of 2.14 × 10^8 CFU/mL *A. veronii* Hm091 based on the previous study (Ran et al., 2018). Sturgeon were challenged with the bacteria for 21 days and at the challenge period fish were not fed. During the challenge period mortality were recorded in every day.

Similarly, GF zebrafish were challenged after three days of colonized with the gut microbiota from sturgeon fed on the SWF® or the basal diet. 30 zebrafish from each treatment group with 6 replications were taken for resistance test of highly pathogenic *A. veronii* Hm091 with the concentration of 2.14 × 10^8 CFU/mL (Ran et al., 2018). Zebrafish were challenged with the bacteria for 2 days and similarly with the sturgeon challenge, no fed were given for zebrafish during challenge period and mortality were recorded at 2 h interval from each treatment and replication tanks.

2.7. Statistical analysis

All the statistical data were presented as values mean with standard deviation (mean ± SEM). All statistical analyses were performed in GraphPad Prism Version 6 software (GraphPad Software Inc. San Diego, CA, USA). Paired t-tests were used to compare the differences between the two groups of data. Differences were considered significant when the p-value was less than 0.05, 0.01 or 0.001, $P < 0.05$(*), $P < 0.01$ (**) or $P < 0.001$ (***).

3. Results

3.1. Growth performance and survival of the hybrid sturgeon

Growth performance and survival rate of sturgeon fed with control diet group or SWF® supplemented diet group were shown (Figs. 1A-E). The results revealed that the weight gain rate of the sturgeon fed on the diet supplemented with SWF® was increased significantly ($P < 0.05$; Fig. 1C) and the FCR was also improved significantly ($P < 0.05$; Fig. 1D), compared with the fish fed on control group. The daily feeding and survival rate of sturgeon were not significantly affected by feeding on the SWF® supplemented diet.

3.2. The resistance of hybrid sturgeon against pathogenic Aeromonus veronii

Fig. 2 presents the effects of dietary SWF® on the resistance of hybrid sturgeon against *A.veronii* Hm091. According to the results, each group of hybrid sturgeon had the highest survival rate during the first 10 days and after 10 days SWF® supplemented diet provoked an increasing trend in the resistance of the hybrid sturgeon against *A. veronii* Hm091, although the difference compared with the control group were not statistically significant ($P = 0.09$).

Table 2
The primer sequences used in the qPCR quantitative analysis for the specific bacteria phyla and total bacteria of sturgeon fed on either experimental diet.

Gene name	Forward primer (5′-3′)	Reverse primer (5′-3′)
Actinobacteria	TACGGCCGCAAGGCTA	TCRTCCCCACCTTCCTCCG
Bacteriodetes	CRAACAGGATTAGATACCCT	GGTAAGGTTCCTCGCGTAT
Firmicutes	GGAGYATGTGGTTTAATTCGAAGCA	AGCTGACGACAACCATGCAC
Fusobacteria	KGGGCTCAACMCMGTATTGCGT	TCGCGTTAGCTTGGGCGCTG
Proteobacteria	TCGTCAGCTCGTGTYGTGA	CGTAAGGGCCATGATG
Total bacteria	CCTACGGGAGGCAGCAG	ATTACCGCGGCTGCTGG

Table 3
The primer sequences of growth promoters and immune regulatory genes of zebrafish for qPCR quantitative analysis.

Gene	Forward primer (5′–3′)	Reverse primer (5′–3′)
mTOR	TGGGAGCAGACAGGAATGAAGG	TGCACCTGCTGGAAAAAGAATG
Myogenin	CGCCGATAATTTCTTCCAGTC	CGTTCACCTTCTTCAACCTCC
MYoD	AGAGGAGGCGACTGAGCAAGGT	CGGTACTGACAGCACGGGACAT
DEFBL1	AGGATGCAGCCTCATTCTCTTT	TGAAGCCCCAGAGCATATTTATC
C3a	ATGAGCTCCTGCAGAGGTGT	AGTGGTTGTTGGAGGTCTGG
Lysozyme	TGGAAGTGGTGTTTTTGTGT	TCAAATCCATCAAGCCCTTC
TGF-β	AGTTGCCTTGTGATTGTGGG	CAATCATATTGGGCACCTGC
IL-1β	GGCTGTGTGTTTGGGAATCT	TGATAAACCAACCGGGACA
TNF-α	GCGCTTTTCTGAATCCTACG	TGCCCAGTCTGTCTCCTTCT

3.3. SWF® supplemented diets alter the gut microbiota of sturgeon

Considering the potential of the gut microbiota to affect growth and disease resistance, we investigated the effects of diet supplementation with SWF® on the gut microbiota abundance of sturgeon, we analyzed and compared the number of bacteria of the most abundance phyla and total bacteria in the gut of hybrid sturgeon by taking gut content sample from the two groups of hybrid sturgeon (Fig. 3A-F). The standard curve developed in the *q*RT-PCR was showed (Supplemental Fig. 1). The R^2 value was 0.996 and indicated that the regression was good. The result showed that in the SWF® group the number of total bacteria, *Fusobacteria, Firmicutes,* and *Proteobacteria* were increased significantly, compared with the control group ($P < 0.05$; Fig. 3A-D), whereas significant reduction in the number of *Actinobacteria* were observed in the gut of the SWF® group compared with the control group ($P < 0.01$; Fig. 3F).

3.4. Gut microbiota of sturgeon induced by SWF® increased the expression of growth related genes of GF zebrafish

We assessed the expression of growth promoter genes in GF zebrafish model colonized by either of the gut microbiota of sturgeon. The relative expression of growth promoter gene, mammalian target of rapamycin (mTOR) was increased significantly ($P < 0.001$) in the SWF®

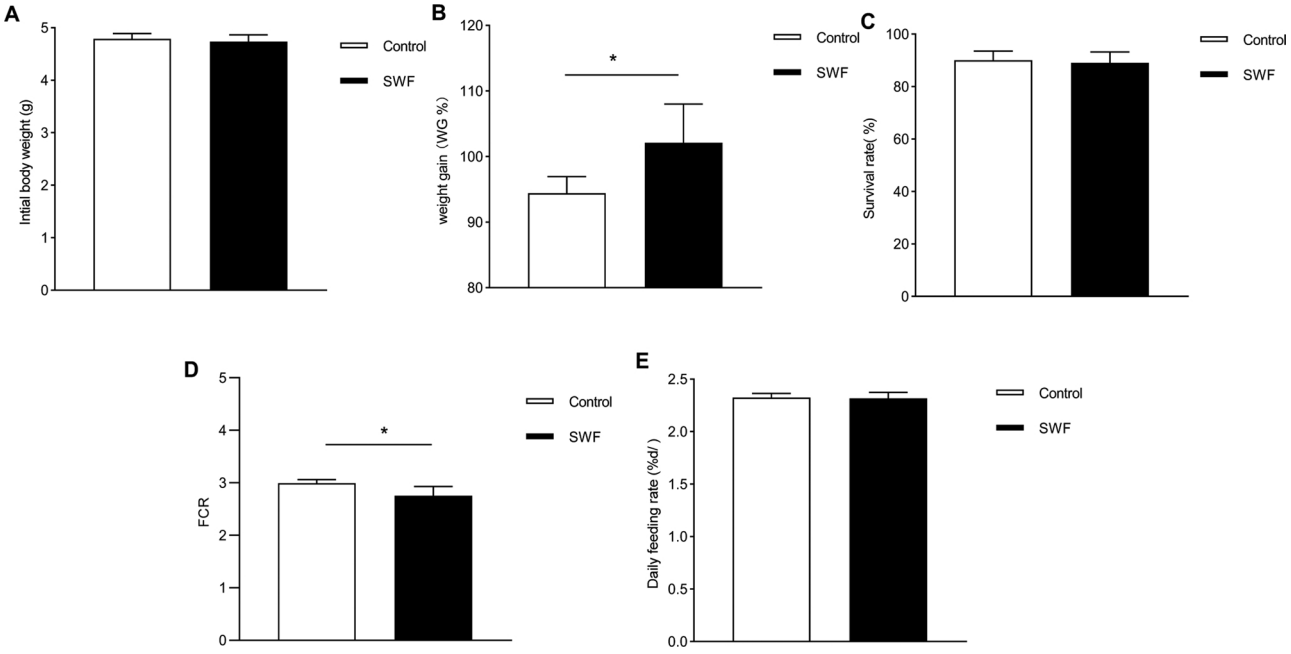

Fig. 1. Effects of SWF® supplemented diet on the weight gain, daily feeding rate, FCR and percentage survival rate of sturgeon.
Percentage weight gain, survival rate, FCR and daily feeding rate of fish fed on one of the two diet groups, data represents the means (± SEM) of six replicates of each treatment, (n = 30/tank) a single asterisk representation of *P*-value < 0.05. (A) Intimal body weight; (B) Percentage weight gain, (C) survival rate; (D), FCR, feed conversion ratio, (E) Daily feeding rate, SWF®, grow worry free.

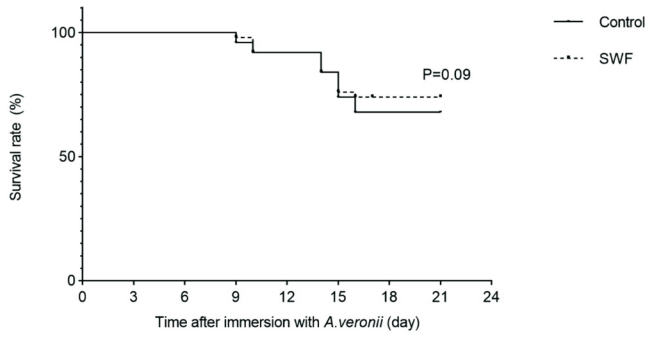

Fig. 2. The effects of SWF® supplemented diet on cumulative survival percentage of sturgeon against pathogenic *Aeromonus veronii*.
Cumulative survival percentage of sturgeon fed on SWF® supplemented diet or control diet when challenged with *A. veronii* Hm091 for 21 days. Each data represents the mean of six replicate tanks (n = 20/tank).

group. Similarly, the expression of myoblast determination protein (MYoD) and Myogenin genes were increased significantly ($P < 0.05$; Fig. 4) in the SWF® group, compared with GF zebrafish group colonized with gut microbiota of sturgeon fed on the basal diet.

3.5. Gut microbiota of sturgeon induced by SWF® increased the resistance of GF zebrafish against pathogenic Aeromonus veronii

In order to further study the effects of gut microbiota taken from hybrid sturgeon fed on either the SWF® supplemented diet or the control diet, GF zebrafish were challenged with highly pathogenic *A. veronii* Hm091 for two days after colonizing with sturgeon gut microbiota for three days. Mortality was counted and recorded in two hours interval. The result revealed that the GF zebrafish model colonized with gut microbiota of sturgeon fed on diet supplemented with SWF® had increased the survival rate significantly against the pathogenic *A. veronii* Hm091 ($P < 0.05$; Fig. 5).

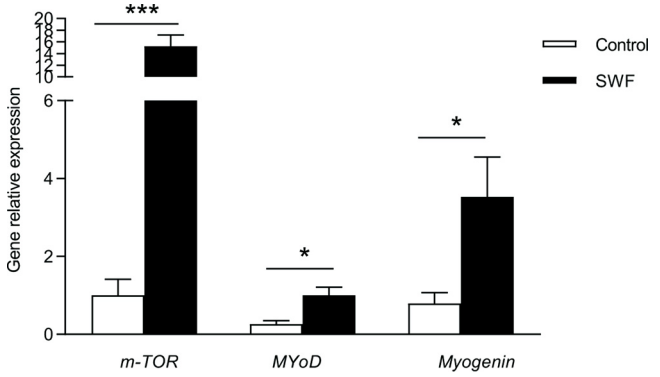

Fig. 4. The effect of SWF® induced gut microbiota of sturgeon on the growth promoter genes of zebrafish.
The effects of gut microbiota of sturgeon on the expression of growth promoter genes of zebrafish, Values are of six replicates of each treatment. The expression levels of genes were assayed in the zebrafish larvae' body after eliminating the head and the viscera. Each data represents the means (± SEM) of six replicate tanks (n = 20/tank), a single and triple asterisk representation of *P*-value < 0.05 and $P < 0.001$, respectively.

3.6. Gut microbiota of sturgeon induced by SWF® enhanced the expression of inflammation and non-specific immunity related genes in GF zebrafish

We hypothesized that beneficial gut microbiota increased the resistance of the fish against pathogenic bacteria might be associated with the regulation of the immune system. Therefore, relative expressions of inflammation and non-specific immunity related genes of GF zebrafish model were assessed. The expression of anti-inflammatory gene TGF-β was significantly increased ($P < 0.01$; Fig. 6A) in GF zebrafish colonized with gut microbiota from sturgeon fed on SWF® supplemented diet, whereas the expression of TNF-α was significantly reduced ($P < 0.05$; Fig. 6A). No significant change where observed in the expression of pro-inflammatory gene IL-1β between the two groups. The

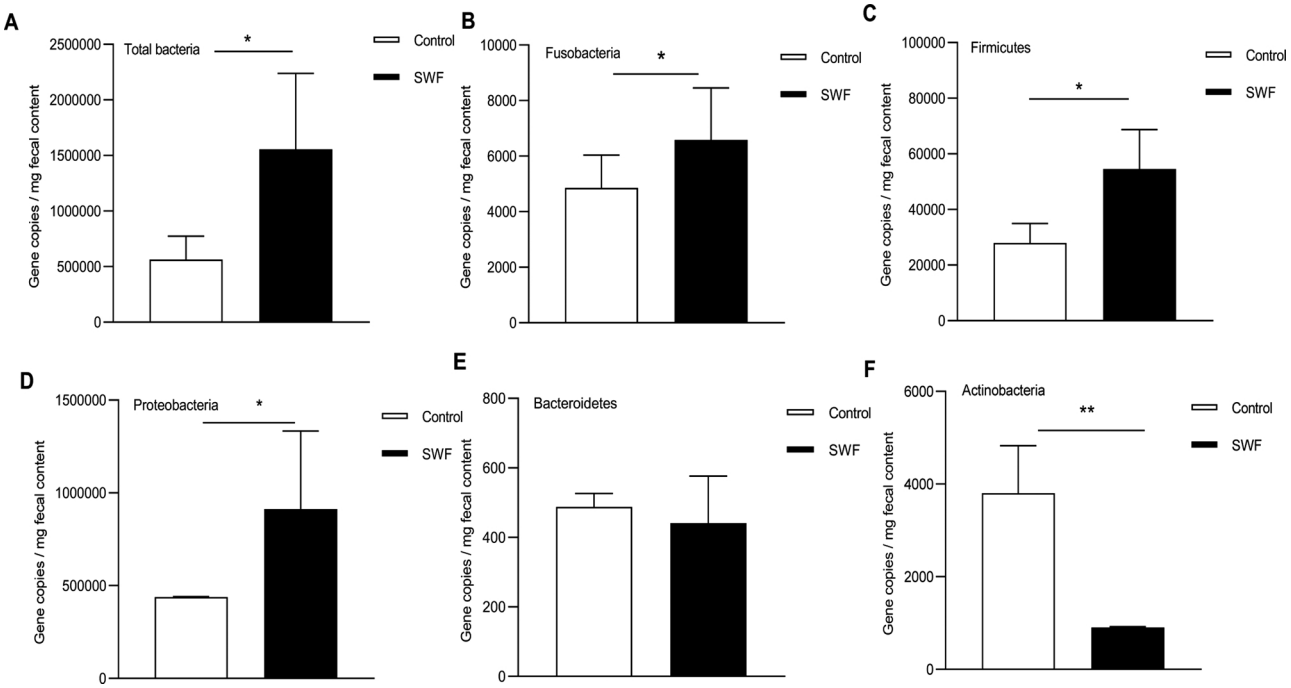

Fig. 3. The effect of SWF® supplemented diet of the number of bacteria of the abundance phyla and total bacteria in the gut of sturgeon.
Data of some bacteria phyla, calculated using 16S rRNA gene copies/mg intestinal contents (A) Total number of bacteria, (B) *Fusobacteria*, (C) *Firmicutes*, (D) *Proteobacteria*, (E) *Bacteriodetes*, (F) *Actinobacteria*, in the intestinal microbiota of hybrid sturgeon, a single and double asterisk representation of *P*-value < 0.05 and $P < 0.01$, respectively.

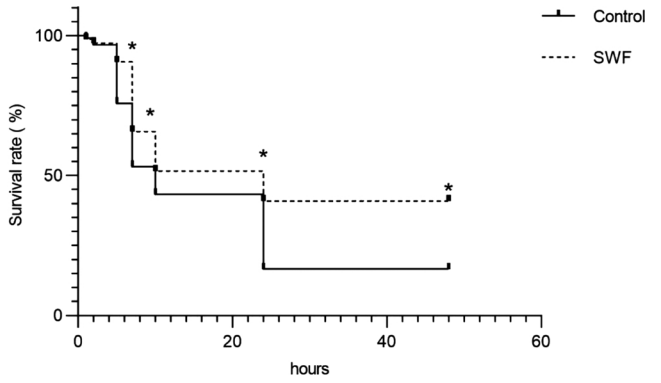

Fig. 5. The effects of SWF® induced gut microbiota of sturgeon on cumulative survival percentage of GF zebrafish against pathogenic *Aeromonus veronii*.
Cumulative survival rates (%) of GF zebrafish colonized with the gut microbiota of sturgeon fed on SWF® supplemented diet or in the control group when challenged with *A. veronii* Hm091 for 2 days. Each data represents the mean of six replicate tanks (n = 30/tank), a single asterisk representation of *P*-value < 0.05.

expression of non-specific immune related gene DEFBL-1 was significantly increased in the GF zebrafish model colonized with the gut microbiota of sturgeon fed with SWF® supplemented diet ($P < 0.01$; Fig. 6B). Furthermore, the expression of C3a and Lysozyme was also increased significantly in the GF zebrafish colonized with gut microbiota from sturgeon fed on SWF® supplemented diet ($P < 0.05$; Fig. 6B).

4. Discussions

The growth and feed utilization of hybrid sturgeon were improved by supplementation of dietary SWF®. Several studies confirmed that live probiotics, killed probiotics or probiotic cell parts and/or metabolites were important to improved growth parameters and feed utilization efficiency of fish by influencing on the gut microbiota (Galagarza et al., 2018; Jaramillo-Torres et al., 2019; Tan et al., 2019). Similar with this results, dietary heat-killed *Lactobacillus plantarum* and β-glucan had a significant interaction on enhancing the growth, digestibility and immune responses of red sea bream (Dawood et al., 2015b). Nile tilapia fed on diet supplemented with *B. pumilus* and the commercial probiotic product Organic Green (Hangpoong Industry Co. Ltd, Korea) for 8 weeks improved weight gain (Mesalhy et al., 2008).

The enhanced growth performance and feed utilization of hybrid sturgeon fed dietary SWF® might be also attributed to the modulation of gut microbiota. In this study, we use the method of absolute quantification of bacteria by a 16S rRNA-based *q*RT-PCR analysis, rather than the 16S rRNA sequencing method. This is because, we can know the change in the absolute amount of the bacteria by the absolute quantitative method (Tkacz et al., 2018). This method also has its weaknesses because of lacking bacterial diversity analysis. However, in this study, we focuse on whether SWF® can alter the gut microbiota and whether SWF® playes its role through gut microbiota. The cell parts and metabolites of probiotic bacteria introduced by SWF® play significant role in promoting the growth of beneficial bacteria in the gut of hybrid sturgeon. Data on this study showed that the number of bacteria in the phyla consists of beneficial bacteria showed significant increased, whereas the number of harmful bacteria was significantly decreased in the SWF® group compared with the control group. In agreement with this result, heat-killed mixed probiotics (*B. subtilis, Lactococcus lactis* and *S. cerevisiae*) significantly reduced the total heterotrophic bacterial population in the intestine of *Labeo rohita* (Mohapatra et al., 2012). This indicated that postbiotics (SWF®) are important to promote the growth of beneficial bacteria and inhibit the pathogenic bacteria. Therefore, SWF® played a great role in the modulation of the gut microbiota of hybrid sturgeon.

Several studies showed that beneficial microbiota have a capacity to modulate the gut microbiota of fish and this modulation results in improvement of body weight via activation of the expression of growth promoter genes (Dawood et al., 2016; Zheng et al., 2017). The mechanisms of the growth of muscle are regulated by the progressive expression of the myogenic regulatory factors family, which includes MyoD, and mygonin (Kinoshita et al., 2011). In this study GF zebrafish colonized with gut microbiota of hybrid sturgeon fed on the SWF® supplemented diet were showed an increased in the expression of Mygonin, MyoD and mTOR genes. *L. vannamei* fed on Poly-β-hydroxybutyrate short chain fatty acid had resulted activation of mTOR signaling and modulated their gut microbiota (Duan et al., 2017).

SWF® supplemented diet provoked an increasing trend in the resistance of the hybrid sturgeon against *A. veronii* Hm091, although the difference compared with the control group were not statistically significant, probably due to the effect of the sample size or short feeding duration (three weeks), because probiotic feeding duration has an effect on disease resistance of fish (Sharifuzzaman and Austin, 2009). Moreover, SWF® may suppress the growth of *A. veronii* Hm091, but did not reduce the mortality of the fish. However, the survival rate of GF zebrafish after challenging with *A. veronii* Hm091 was improved due to colonization of gut microbiota from the hybrid sturgeon fed on the SWF® supplemented diet. Because the immune system of GF zebrafish is

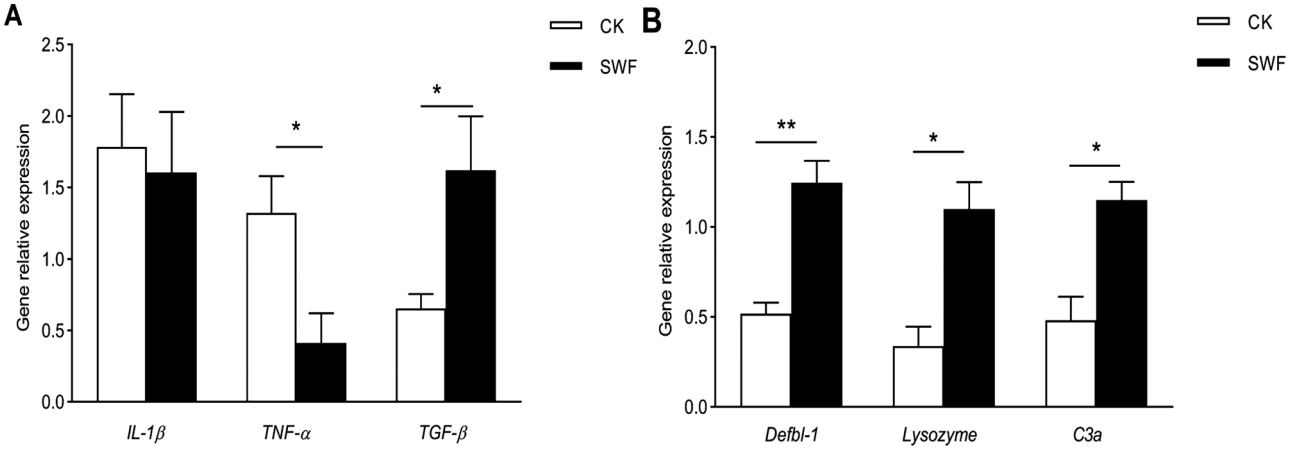

Fig. 6. The effect of SWF® induced gut microbiota of sturgeon on the inflammatory and immune regulatory genes of GF zebrafish.
The effects of gut microbiota of sturgeon on the expression of (A) inflammatory related genes, (B) immune regulatory genes of GF zebrafish, values are of six replicate tanks of each treatment (n = 20/tank). The expression levels of genes were assayed in the zebrafish larvae' body after eliminating the head and the viscera. Each data represents the means (± SEM) of six replicates, a single asterisk representation of *P*-value < 0.05, double asterisk representation of *P*-value < 0.01.

lower than the conventional fish, even though the GF zebrafish were colonized with gut microbiota of hybrid sturgeon for short period of time (three days). Because studies demonstrated that zebrafish innate immune development is regulated by the presence of gut microbiota (Galindo-Villegas et al., 2012). The increase in the expression of non-specific immunity and some anti-inflammatory related genes may have cumulatively resulted in higher survival against *A. veronni* Hm091 in the SWF® compared to the control group. Supplementation with heat killed probiotics or probiotic cell components have demonstrated increased disease resistance against infectious *S. iniae* and *Yersinia ruckeri* challenges in rainbow trout (Abbass et al., 2010; Brunt and Austin, 2005). Pan et al. (2008), also revealed dead or live *Clostridium butyrium* CB2 improved immune response and enhanced disease resistance in Chinese drum (*Miichthys miiuy*). The improvement of survival rate of zebrafish to *A. veronii* Hm091 challenges can be attributed to better immunological status the gut microbiota of the sturgeon fed on the SWF® containing diet.

Furthermore, our finding showed that, the expression of non-specific immune related genes including DEFBL-1, Lysozyme and C3a were increased in GF zebrafish group colonized with gut microbiota of hybrid sturgeon fed on the SWF® amended diet. This indicated that the modulated gut microbiota of sturgeon via the SWF®, was contribute for the improvement of immune system of GF zebrafish model, since normal gut microbiota contributes indispensable roles in regulating the fish immune system, and vice versa. Consistent with this result, Mohammadian et al. (2018), revealed that the activities of lysozyme and C3 genes were significantly increased in *Tor grypus* fish fed on diet supplemented with probiotics. In addition to this, C3a gene expression was increased in zebrafish treated with *L. casei BL23* after challenged with *A. hydrophila* (Xie et al., 2018). TGF-β is a potent immune-deviating cytokine with pivotal roles in inducing active immune tolerance in mucosal and peripheral tissues (Sanjabi et al., 2017). The expression of anti-inflammatory related gene TGF-β was increased in GF zebrafish colonized with the gut microbiota of sturgeon fed on the SWF® supplemented diet. In agreement with this study, *O. mykiss* and hybrid tilapia fed on different types of probiotics were resulted an increased the expression of several cytokines genes including, TGF-β (He et al., 2017; Panigrahi et al., 2011, 2007). Inactivated probiotic bacteria *B. amyloliquefaciens* FPTB16 and *B. subtilis* FPTB13 stimulate cellular immune responses of *Catla catla* (Kamilya et al., 2015). The expression of pro-inflammatory genes TNF-α and IL-1β were decreased in zebrafish colonized with gut microbiota from SWF® group. In line with this study (Wu, 2020) demonstrated that grass carp (*Ctenopharyngodon idella*) fed on the diet supplemented with *B. licheniformis* FA6 exhibited a decreased mRNA expression of pro-inflammatory cytokines (IL-1β and TNF-α).

5. Conclusions

In conclusion, beneficial bacteria play significant role in modulation of the gut microbiota of the fish. Our fining showed that dietary SWF® resulted in improvement of weight gain and feed conversion ratio. There was an improvement in survival rate of hybrid sturgeon fed on SWF® amended diet against *A. veronii* Hm091 ($P = 0.09$). Dietary SWF® altered the gut bacteria of hybrid sturgeon. Besides, our result showed the expression of grow promoter, inflammation and non-specific immune regulation related genes was improved in the GF zebrafish model colonized with the gut microbiota of sturgeon fed on the diet supplemented with SWF®. Likewise, the survival rate of zebrafish challenged with highly pathogenic *A. veronii* Hm091 was improved in the group of GF zebrafish colonized with gut microbiota of sturgeon fed on the diet supplemented with SWF®. Taken together, our study indicated that feeding fish with SWF® improved the growth, feed utilization and disease resistance of the hybrid sturgeon via modulation and stabilization of the gut microbiota of the fish and could be considered as potent therapeutic agents and play significant role in improvement of the production and productivity of aquaculture in the post-antibiotic era.

Declaration of competing interest

The authors declare that they have no conflict of interest.

Author statement

The authors' responsibilities were as follows: Zhigang Zhou and Zhen Zhang designed research. Tsegay Teame and Zhen Zhang wrote the manuscript with contributions of all other authors especially with regard to their part of the work; Youming Zhang, Chao Ran, Yalin Yang, Liqiu Xia, and Shaojun Wei gave conceptual advice for the manuscript. Xuexiang Wu and Qiang Hao performed experiments and acquired data. Qianwen Ding helped to edit the manuscript and statistical analysis. Hongliang Liu helped in sampling of fish. All authors read, commented on and approved the final manuscript.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.aqrep.2020.100346.

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